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Alternative route for the biosynthesis of polyunsaturated fatty acids in K562 cells

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K562 human leukaemia cells lack a significant Δ^6-desaturase activity. However, they synthesize long-chain polyunsaturated fatty acids (PUFA) from linoleic ($C_{18:2(9,12)}$) and linolenic ($C_{18:3(9,12,15)}$) acids, by reactions involving a C_2 chain elongation followed by a Δ^5-desaturation step and, to some extent, a further elongation. The main products formed were separated by argentation t.l.c. and identified by g.l.c. as the uncommon fatty acids $C_{20:3(5,11,14)}$ and $C_{20:4(5,11,14,17)}$ respectively. These acids were also produced when cells were supplemented with $C_{20:2(11,14)}$ or $C_{20:3(11,14,17)}$ respectively. The presence of a Δ^5-desaturase was further confirmed by using its corresponding normal substrates, $C_{20:3(8,11,14)}$ and $C_{20:4(8,11,14,17)}$, which led to $C_{20:4(5,8,11,14)}$ and $C_{20:5(5,8,11,14,17)}$ respectively. On the other hand, a high Δ^9-desaturase activity, but no significant Δ^4-desaturase activity, were detected in K562 cells. These results indicate the existence of an alternative pathway, involving Δ^5-desaturase, which is the only route for PUFA biosynthesis in K562 cells. This pathway may be relevant for the biosynthesis of PUFA in cells lacking Δ^6-desaturase activity.

INTRODUCTION

Biosynthesis of long-chain polyunsaturated fatty acids (PUFA) is known to occur through desaturation and elongation reactions from the essential fatty acids linoleic ($C_{18:2,n-6}$) and linolenic ($C_{18:3,n-3}$). The pathway involves the sequential action of several desaturases (Δ^6, Δ^5 and Δ^4, in this order) alternating their action with those of enzymes of C_2 chain elongation. This classical pathway is considered to be universal and acts on the precursors and intermediates of the $n-6$ and $n-3$ families of PUFA. The limiting step is usually the Δ^6 desaturation, which determines the capacity of a particular cell for PUFA biosynthesis. This enzyme is more active on $C_{18:3,n-3}$ than on $C_{18:2,n-6}$, and the relative amounts of both precursors determine the type of PUFA formed (Cook, 1985; Rosenthal, 1987).

However, recent work by Cook et al. (1991) suggests the existence of an alternative pathway of $C_{18:3,n-3}$ desaturation in C6 rat glioma cells, operating simultaneously with the classical pathway. This new route essentially involves the elongation of $C_{18:3}$ to $C_{20:3}$ and then a Δ^5-desaturation step to form the unusual fatty acid $C_{20:4(5,11,14,17)}$, which can be further elongated to $C_{22:4(7,13,16,19)}$ (Cook et al., 1991). In the present work, we have found that in the human leukaemic cell line K562 this alternative route, involving Δ^5-desaturase, is the only operative pathway. This pathway may operate on both the $n-3$ and $n-6$ families of PUFA, and, like the classical pathway, shows greater activity on the fatty acids of the $n-3$ series.

MATERIALS AND METHODS

Cells

K562 cells were initially classified as a human erythroleukaemia, and more recently as multipotential malignant haematopoietic cells (Hay et al., 1988). Cells (mycoplasma-free) were kindly provided by Dr. Jeremy Brock (Department of Immunology, University of Glasgow, U.K.) and grown in 25 cm^2 or 75 cm^2 flasks (Nunc) in RPMI 1640 medium supplemented with 10% fetal-calf serum (FCS), glutamine (1 mM) and antibiotics (penicillin/streptomycin). Cells were seeded at a density of $(1.5–2) \times 10^5$ cells/ml and routinely propagated each 72–96 h.

Materials

Seeds from *Cimicifuga racemosa* were a gift from Dr. Edward A. Emken (USDA–ARS, Northern Regional Research Center, Peoria, IL, U.S.A.). Fatty acid-free BSA and the non-radioactive fatty acids $C_{17:0}$ (n-heptadecanoic), $C_{18:0}$ (stearic), $C_{18:2,n-6}$ (linoleic or octadeca-9,12-dienoic acid), $C_{18:3,n-3}$ (α-linolenic or octadeca-9,12,15-trienoic acid), $C_{18:3,n-6}$ (γ-linolenic or octadeca-6,9,12-trienoic acid), $C_{18:4,n-3}$ (octadeca-6,9,12,15-tetraenoic acid), $C_{20:2,n-6}$ (eicosa-11,14-dienoic acid), $C_{20:3,n-6}$ (eicosa-8,11,14-trienoic acid), $C_{20:3,n-3}$ (eicosa-11,14,17-trienoic acid), $C_{20:4,n-6}$ (arachidonic or eicosa-5,8,11,14-tetraenoic acid) and $C_{20:5,n-3}$ (eicosa-5,8,11,14,17-pentaenoic acid) were obtained from Sigma (U.K.). 1-^{14}C-labelled stearic, linoleic and α-linolenic acids were products from NEN (Itisa, Spain). Radiochemical purity, determined by argentation t.l.c., was > 98%. In all cases, fatty acids were dissolved in toluene, and stock solutions (5–10 mg/ml) were stored under nitrogen at −30 °C until their use. Silica gel G60 was from Merck (Igoda, Spain).

Evaluation of desaturase activities

Two experimental protocols were used to characterize the fatty acid desaturation pathways in K562 cells. In the first protocol, 1 μCi of [1-^{14}C]stearic, [1-^{14}C]linoleic or [1-^{14}C]linolenic acid (equivalent to 14, 20 and 23 nmol respectively) bound to fatty acid-free BSA (1:1 molar ratio) was added to cell cultures 48 h after seeding and incubated for another 3 or 24 h (during the exponential phase of cell growth). Then cells were separated from media by centrifugation at 4 °C (200 *g*, 10 min) and washed three times by resuspension in ice-cold RPMI and centrifugation. The resulting cell pellet was used for lipid extraction and analysis.

In the second protocol, cells were supplemented with different fatty acid precursors ($C_{18:0}$, $C_{18:2,n-6}$, $C_{18:3,n-3}$, $C_{18:4,n-3}$, $C_{20:2,n-6}$,

Abbreviations used: FCS, fetal-calf serum; PUFA, polyunsaturated fatty acids.

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Table 1 Non-esterified fatty acid composition of the FCS used in experiments

Non-esterified fatty acids were extracted from serum as described in the Materials and methods section. Data are expressed as weight per cent of the total acids and as μg of fatty acid per ml of culture medium.

Fatty acid	Amount (% of total)	Concn. (μg/ml)
$C_{16:0}$	44.6	1.34
$C_{16:1,n-7}$	4.4	0.13
$C_{18:0}$	17.5	0.53
$C_{18:1,n-9}$	18.4	0.55
$C_{18:2,n-6}$	6.3	0.19
$C_{20:4,n-6}$	8.7	0.26
Total	100.0	3.0

Table 2 Fatty acid composition of lipids from *Cimicifuga racemosa* seeds

Fatty acids were extracted from seeds and analysed as described in the Materials and methods section. Data are expressed as weight percent of the total fatty acids.

Fatty acid	Amount (% of total)
$C_{16:0}$	7.4
$C_{16:1,n-7}$	1.7
$C_{18:0}$	2.8
$C_{18:1,n-9}$	8.2
$C_{18:2,n-6}$	29.7
$C_{18:3,n-3}$	7.4
$C_{20:1,n-9}$	19.9
$C_{20:2,n-6}$	5.8
$C_{20:3(5,11,14)}$	7.1
$C_{20:3,n-3}$	2.6
$C_{20:4(5,11,14,17)}$	7.3
$C_{24:0}$	0.1

$C_{20:3,n-6}$, $C_{20:3,n-3}$ or $C_{20:5,n-3}$). Fatty acid-supplemented media were prepared as described in detail in Anel et al. (1992). In brief, portions of stock toluene solutions of each fatty acid were transferred to screw-cap glass tubes. The solvent was evaporated under nitrogen and the appropriate amount of FCS was added. The mixture was incubated at 37 °C for 1 h, sterilized through a 0.22 μm-pore Millipore filter (Millex-GV), and the actual amount of fatty acids bound to serum proteins was determined by g.l.c. The fatty acids complexed to FCS were added to the culture media at a final concentration of 20–30 μM. In these conditions, the molar ratio fatty acids/carrier proteins (mainly albumin) was $\leqslant 1.5$. Cells were cultured in supplemented media (containing 10% FCS) for 40 h, harvested and washed three times by resuspension in ice-cold RPMI and centrifugation, before lipid extraction. Fatty acids from total cell lipids were analysed by g.l.c., either unfractionated or after fractionation by argentation t.l.c. (see under 'Lipid analysis').

Lipid analysis

Cell lipids were extracted by vigorous shaking in chloroform/methanol (2:1, v/v), as described by Anel et al. (1990). Fatty acid methyl esters, from total lipids of supplemented and control cells, were prepared by reaction at 80 °C, under N_2, with 5% H_2SO_4 in anhydrous methanol for 1.5 h. Small amounts of antioxidant (2,6-di-t-butyl-4-methylphenol; 'BHT'; 2 μg/10^6 cells) and internal standard ($C_{17:0}$; 2 μg/10^6 cells) were added. To determine the amount and composition of the non-esterified fatty acids bound to serum proteins in control (Table 1) and supplemented media, lipids from the different serum samples were also extracted with chloroform/methanol (2:1, v/v). Non-esterified fatty acids were separated from the other lipid classes by t.l.c. on silica gel G plates, with hexane/diethyl ether/acetic acid (70:30:1, by vol.) as eluent. The plates were developed with 2′,7′-dichlorofluorescein (0.2% in methanol), stained areas scraped off and fatty acids transmethylated as described above. Fatty acid methyl esters were analysed in a Shimadzu GC9A gas chromatograph, equipped with a column (2 m × 3.2 mm) of 10% SP-2330 on Chromosorb WAW (Supelco, U.S.A.), a flame ionization detector and a C-R3A data processor–integrator. Helium (30 ml/min) was used as carrier gas. The initial column temperature was 185 °C and was increased after 14 min to 195 °C, at 1 °C/min, and held until 60 min. The injector and detector temperatures were 250 °C. Fatty acids, except those generated in the alternative pathway, were identified by comparing their retention times with those of pure standards. Lipids from seeds of *Cimicifuga racemosa* were extracted with chloroform/methanol (2:1, v/v) and trans-esterified as indicated for cells. The fatty acid composition of seed lipids is indicated in Table 2.

In desaturation experiments, fatty acid methyl esters from total cell lipids were fractionated according to their degree of unsaturation, by argentation t.l.c. (Christie, 1982). The preparation of plates and the solvent systems have been described in detail previously (Anel et al., 1990). Fractions were detected under u.v. light with 2′,7′-dichlorofluorescein, scraped off, and radioactivity was determined by liquid-scintillation counting. The resolution of the method was checked by adding a known amount of pure fatty acid standards in the same plates as for cell lipid samples, and by adding a known amount of fatty acid standards to cell samples containing radiolabelled fatty acids and analysing the stained bands by g.l.c. The identity of fatty acid desaturation products was further confirmed by separating fatty acid methyl esters from total lipids, of control and fatty acid-supplemented cells, by argentation t.l.c. and analysing the separated fractions by g.l.c. Methyl esters obtained from the seed lipids of *Cimicifuga racemosa* were used to determine g.l.c. retention times for $C_{20:3(5,11,14)}$ and $C_{20:4(5,11,14,17)}$. Seeds from *Cimicifuga racemosa* contain significant amounts of these fatty acids (Table 2, and E. A. Emken, personal communication), initially identified in *Ephedra campylopoda* (Cook et al., 1991). Further confirmation of the identity of alternative fatty acids from K562 cells was performed by combined g.l.c./chemical-ionization m.s. analysis. Fatty acid methyl esters from *Cimicifuga racemosa* seeds and from K562 cells supplemented with $C_{20:2,n-6}$ or $C_{20:3,n-3}$ were separated at 190 °C on a SP-2330 (Supelco) capillary column (30 m × 0.25 mm, film thickness 0.2 μm) directly inserted into the source of the mass spectrometer (VG AutoSpec; VG Analytical, Manchester, U.K.). The ion source temperature was 150 °C and methane was used as chemical-ionization reagent.

Statistical significance of the variations of fatty acid composition was analysed by Student's *t* test for non-paired variates, by using the StatView software for Apple Macintosh computers.

RESULTS

K562 cells incubated for 24 h with radiolabelled $C_{18:0}$ converted more than 40% of the incorporated fatty acid into monoenes (equivalent to around 440 pmol/10^6 cells), indicating the exist-

Table 3 Desaturation of $C_{18:2,n-6}$ and $C_{18:3,n-3}$ by K562 cells

Cells in the exponential phase of growth ($\sim 4 \times 10^6$ in 10 ml of culture medium) were cultured with 1 μCi of the corresponding radiolabelled fatty acid for 3 h or 24 h, harvested and total radioactivity incorporated was determined. Total cell lipids were extracted with chloroform/methanol and fatty acids were transmethylated with H_2SO_4 in methanol. Fatty acid methyl esters were separated according to their degree of unsaturation by argentation t.l.c. Data are means of duplicate determinations on three independent cultures. The total conversion of each fatty acid precursor was calculated by considering the amount incorporated into cell lipids. The concentration in media of these fatty acids (in non-esterified form) is shown in Table 1; nd, not detected.

	Radioactivity distribution (% of total incorporation) in cells incubated with:			
	$[^{14}C]C_{18:2}$		$[^{14}C]C_{18:3}$	
Fatty acid type	3 h	24 h	3 h	24 h
Dienes	97.2	97.0	–	–
Trienes	2.8	2.9	85.6	77.0
Tetraenes	0.1	0.1	12.7	22.5
Pentaenes	nd	nd	0.2	0.3
Hexaenes	nd	nd	0.2	0.2
Total conversion (pmol of fatty acid/10^6 cells)	38.6	63.0	89.7	444.1

Table 4 Fatty acid composition of total lipids from K562 cells supplemented with different fatty acids

K562 cells were cultured for 40 h in control medium or in media supplemented with 30 μM concentration of stearic ($C_{18:0}$), linoleic ($C_{18:2}$) or linolenic ($C_{18:3}$) acid. Results are expressed as weight per cent of total fatty acids and are means of two individual determinations on three separate cultures. S.D. was always less than 10% of the mean. Abbreviations: DMA, dimethyl acetal of alkenyl side chains of plasmalogens; nd, not detected.

Fatty acid	Controls	$+C_{18:0}$	$+C_{18:2,n-6}$	$+C_{18:3,n-3}$
$C_{16:0}$ DMA	1.6	0.9	0.6	nd
$C_{16:0}$	17.9	15.3	21.3	23.2
$C_{16:1,n-7}$	2.3	2.5	2.1	2.0
$C_{18:0}$	18.7	21.3	10.7	11.1
$C_{18:1,n-9}$	39.6	43.7	23.0	27.3
$C_{18:2,n-6}$	1.6	1.5	28.1	1.8
$C_{18:3,n-3}$	0.6	0.9	0.2	12.2
$C_{20:1,n-9}$	3.1	3.3	1.2	1.1
$C_{20:2,n-6}$	0.9	nd	5.1	nd
$C_{20:3(8,11,14)}$	0.5	0.3	nd	nd
$C_{20:3(5,11,14)}$	nd	nd	1.7	nd
$C_{20:3,n-3}+C_{20:4,n-6}$*	4.8	4.0	2.3	9.0
$C_{20:4(5,11,14,17)}$	0.6	nd	nd	4.4
$C_{20:5,n-3}$	0.6	0.9	nd	0.4
$C_{22:1,n-9}$	0.6	0.4	0.4	nd
$C_{22:3(7,13,16)}$	nd	nd	0.4	nd
$C_{22:4,n-6}$	1.3	0.2	1.0	1.8
$C_{22:4(7,13,16,19)}$	nd	nd	nd	2.1
$C_{22:5,n-3}$	2.1	2.4	1.0	1.6
$C_{22:6,n-3}$	2.3	1.7	0.9	1.8
Fatty acids (μg/10^6 cells)	13.1	19.6	36.3	24.7

* Peaks not resolved by our g.l.c. column.

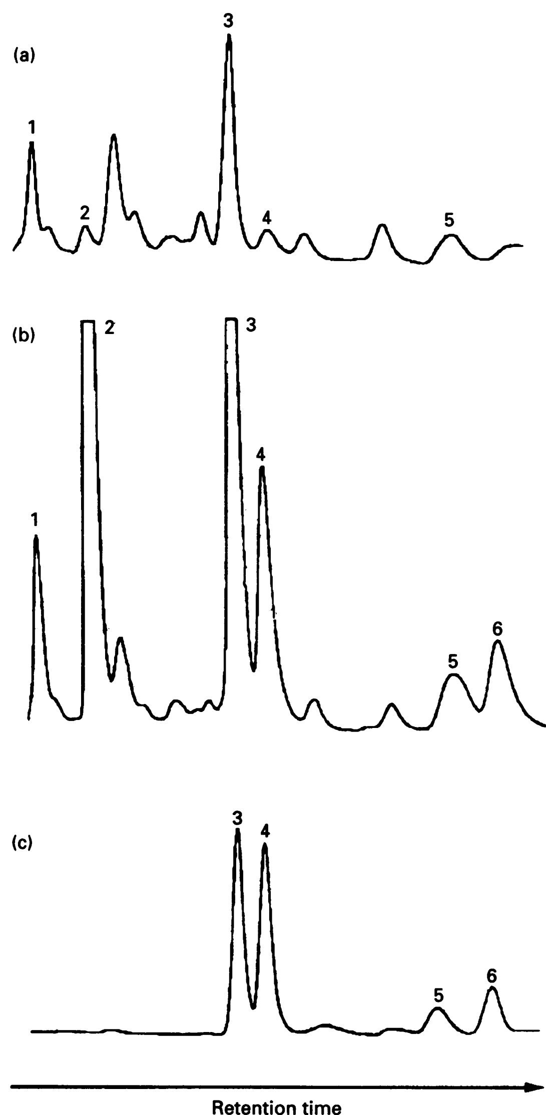

Figure 1 Portions of g.l.c. profiles of fatty acids from K562 cells

K562 cells were cultured for 40 h in control medium (**a**) or in media supplemented with 30 μM linolenic acid ($C_{18:3,n-3}$) (**b**, **c**) and fatty acid methyl esters were obtained from total cell lipids. Methyl esters were analysed by g.l.c. as indicated in the Materials and methods section. The fatty acid methyl esters from cells in (**b**) were separated by argentation t.l.c. and the tetraenes fraction was analysed by g.l.c., as shown in (**c**). Approximate retention times for all chromatograms were 12–44 min. Peaks: 1, $C_{18:2(9,12)}$; 2, $C_{18:3(9,12,15)}$; 3, $C_{20:3(11,14,17)}+C_{20:4(5,8,11,14)}$; 4, $C_{20:4(5,11,14,17)}$; 5, $C_{22:4(7,10,13,16)}$; 6, $C_{22:4(7,13,16,19)}$.

ence of a Δ^9-desaturase activity. After a 24 h incubation, cells incorporated approx. 10.8 nmol of $C_{18:2}$ and 10.4 nmol of $C_{18:3}$ in cell lipids, and around 3% and 23% of these amounts, respectively, were converted into more unsaturated fatty acids (Table 3). Unexpectedly, nearly all the desaturation products of $C_{18:2}$ were recovered in the trienes fraction and those of $C_{18:3}$ in tetraenes, suggesting the existence of a single type of desaturase activity, acting on both fatty acids. To characterize these main products, cells were supplemented with $C_{18:0}$, $C_{18:2}$ or $C_{18:3}$, and the changes in fatty acid composition of cell lipids analysed (Table 4). Incubation with $C_{18:0}$ caused a significant ($P < 0.05$) increase in the proportion of $C_{18:1,n-9}$. In cells supplemented with $C_{18:2,n-6}$ the proportion of $C_{20:2}$, its main elongation product, greatly increased and two new fatty acids, tentatively identified by g.l.c. as $C_{20:3(5,11,14)}$ and $C_{22:3(7,13,16)}$, appeared (Table 4). An analogous phenomenon was more clearly observed in supplementations with $C_{18:3,n-3}$ (Figure 1b), which led to a increase in $C_{20:3,n-3}$ (elongation product) and to the generation of the unusual

Table 5 Effect of supplementation with different intermediates of the biosynthesis of PUFA on fatty acid composition of K562 cells

Cells were cultured in control medium or media supplemented with the fatty acids indicated for 40 h. The fatty acid concentration was 30 μM for $C_{18:4,n-3}$ and $C_{20:2,n-6}$ and 20 μM for the rest of fatty acids. Results are expressed as weight per cent of total fatty acids and represent the mean of three individual determinations on four separate cultures. S.D. was never greater than 15% of the mean.

Fatty acid	Controls	$+C_{18:4,n-3}$	$+C_{20:2,n-6}$	$+C_{20:3,n-3}$	$+C_{20:3,n-6}$	$+C_{20:5,n-3}$
$C_{18:2,n-6}$	1.59	1.41	3.10	1.45	1.38	2.01
$C_{18:3,n-3}$	0.61	0.35	0.24	0.78	0.41	nd
$C_{18:4,n-3}$	nd	2.68	0.93	nd	nd	nd
$C_{20:3(5,11,14)}$	nd	nd	4.53	nd	nd	nd
$C_{20:3(8,11,14)}$	0.54	0.56	nd	0.49	3.34	nd
$C_{20:3,n-3}+C_{20:4,n-6}$	4.85	3.37	2.71	26.70	7.90	2.85
$C_{20:4(5,11,14,17)}$	0.65	nd	nd	7.41	nd	nd
$C_{20:4(8,11,14,17)}$	nd	16.18	nd	nd	0.64	0.42
$C_{20:5,n-3}$	0.60	5.28	1.17	nd	nd	9.00
$C_{22:3,n-6}$	nd	nd	nd	nd	1.20	nd
$C_{22:4,n-6}$	1.30	0.81	0.48	2.16	3.41	1.50
$C_{22:4(7,13,16,19)}$	nd	nd	nd	2.85	nd	nd
$C_{22:4(10,13,16,19)}$	nd	0.90	nd	nd	nd	nd
$C_{22:5,n-3}$	2.10	4.47	1.46	1.90	2.43	15.48
$C_{22:6,n-3}$	2.35	1.49	1.46	1.81	2.19	1.39

fatty acids $C_{20:4(5,11,14,17)}$ and $C_{22:4(7,13,16,19)}$. These fatty acids appeared in the tetraene band when separated by argentation t.l.c. (Figure 1c), and were eluted on g.l.c. with a retention time slightly shorter than that of their isomers from the classical pathway (compatible with a shift due to a Δ^5-double bond instead of a Δ^8-double bond). In addition, the fatty acids identified as $C_{20:3(5,11,14)}$ and $C_{20:4(5,11,14,17)}$ were eluted with retention times coincident with the corresponding fatty acids present in *Cimicifuga racemosa* seed lipids (Table 2, and E. A. Emken, personal communication). These results suggested the existence of a Δ^5-desaturase activity, acting on the elongation products of $C_{18:2,n-6}$ and $C_{18:3,n-3}$. On the other hand, the proportion of arachidonic and eicosapentaenoic acid, the normal products of the combined activity of Δ^6- and Δ^5-desaturases, decreased in supplemented cells (Table 4), all of which is compatible with the absence of a Δ^6-desaturase activity.

The identity of these fatty acids was further confirmed by supplementing K562 cells with different intermediates of the biosynthesis of PUFA (Table 5). The existence of a Δ^5-desaturase was confirmed by incubating cells with $C_{18:4,n-3}$, a substrate that by-passes the Δ^6-desaturation step in the classical pathway. This supplementation leads to an increase in the amounts of cellular $C_{20:4,n-3}$ (elongation product) and $C_{20:5,n-3}$ and $C_{22:5,n-3}$ (desaturation products). However, supplementation with $C_{20:3,n-3}$ caused the appearance of the unusual fatty acid $C_{20:4(5,11,14,17)}$ and its elongation product $C_{22:4(7,13,16,19)}$. These fatty acids were recovered in the tetraenes fraction of argentation t.l.c. plates (results not shown). Similarly, cells incubated with $C_{20:3,n-6}$, a normal substrate of Δ^5-desaturase, increased their content of arachidonic acid ($C_{20:4}$), and when K562 cells were supplemented with $C_{20:2(11,14)}$, it was converted into $C_{20:3(5,11,14)}$. In this case, a small part of the $C_{20:2}$ incorporated was converted back into $C_{18:2}$ (Table 5). The supplementation of cultures with $C_{20:5,n-3}$ caused an increase in the proportion of this fatty acid and that of its elongation product ($C_{22:5}$) in cell lipids, but the amount of $C_{22:6}$ decreased significantly ($P < 0.05$), suggesting a lack of Δ^4-desaturase activity in K562 cells.

The fatty acid methyl esters from K562 cell lipids and *Cimicifuga racemosa* seed lipids were also analysed by combined g.l.c./chemical-ionization m.s. The mass spectra of $C_{20:3(5,11,14)}$ and $C_{20:4(5,11,14,17)}$ from either *Cimicifuga racemosa* or K562 cells

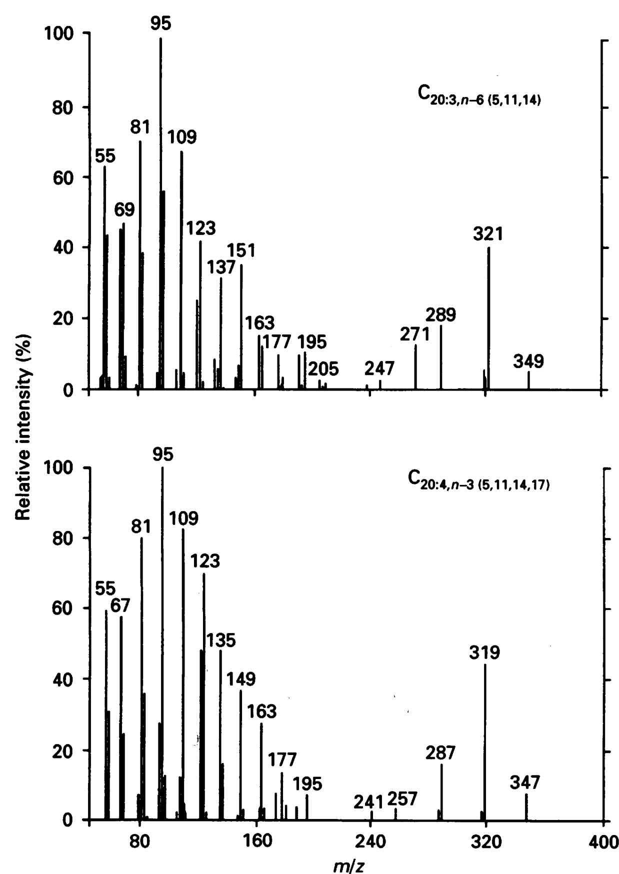

Figure 2 Chemical-ionization m.s. of alternative fatty acids

Combined g.l.c./chemical-ionization m.s. of fatty acid methyl esters from K562 cell lipids was carried out as described in the text. The spectra shown correspond to $C_{20:3(5,11,14)}$ (top) and $C_{20:4(5,11,14,17)}$ (bottom).

were virtually identical, and those from K562 cells are shown in Figure 2. The molecular $[M+H^+]$ ions appeared at m/z 321 and 319 respectively, as well as, in both spectra, the characteristics

$[M+29]^+$ (addition of $C_2H_5^+$) and $[M+H-32]^+$ (loss of methanol), which confirmed their carbon chain length and degree of unsaturation. The relative abundance of ions at m/z 109 and m/z 151 in each spectrum permits the assignment of $C_{20:3}$ to the $n-6$ and $C_{20:4}$ to the $n-3$ series (Fellenberg et al., 1987).

DISCUSSION

This work was undertaken as part of a more general study on the metabolism of unsaturated fatty acid in human lymphoid cells. We have analysed the activity of desaturases during blastic transformation of T-lymphocytes (Anel et al., 1990), and we are now investigating the desaturation–elongation pathways in different leukaemia and lymphoma cells. Most cells studied up to now have shown desaturation activities according to the classical pathway described for the synthesis of the $n-6$ and $n-3$ series of PUFA (J. Naval, I. Marzo, M. Martínez-Lorenzo and A. Piñeiro, unpublished work). This pathway proceeds by an alternating sequence of desaturation and chain-elongation reactions involving the sequential action of Δ^6, Δ^5- and Δ^4-desaturases (Cook, 1985; Rosenthal, 1987). Recently, Cook et al. (1991, 1992), using deuterated substrates, have suggested the existence of an alternative pathway of $C_{18:3,n-3}$ desaturation in C6 rat glioma cells, operating simultaneously with the classical pathway. This new route consists essentially of the elongation of $C_{18:3}$ to $C_{20:3}$, followed by a Δ^5-desaturation step to form the unusual acid $C_{20:4(5,11,14,17)}$, which may be further elongated to form $C_{22:4(7,13,16,19)}$. We have now found that in the human leukaemic cell line K562, which does not show a Δ^6-desaturase activity, this alternative route, involving Δ^5-desaturase, is the only operative pathway. In these cells, the pathway is operative for both the $n-6$ and $n-3$ families of PUFA, and like the classical pathway, shows greater activity on fatty acids of the $n-3$ than of the $n-6$ series. The fatty acids biosynthesized were identified on the basis of their degree of unsaturation (by argentation t.l.c.), data from fatty acid supplementation experiments, the coincidence of their g.l.c. retention time and mass spectra with those of the corresponding fatty acids from *Cimicifuga racemosa*, and the data on molecular mass and double-bond positional information from m.s. analysis.

The occurrence of this alternative pathway is probably more general than at present recognized, although in cells possessing both Δ^6- and Δ^5-desaturase activities this pathway would be only of minor importance. Even if cells were supplemented with the fatty acids that may act as substrates for the Δ^5-desaturase in the alternative pathway ($C_{20:2,n-6}$ or $C_{20:3,n-3}$), this route could be obscured by the effect of retroconversion that regenerates the substrates of the normal pathway (Maeda et al., 1978; Rosenthal, 1987). However, this route could be relevant in cells lacking Δ^6- and possessing Δ^5-desaturase activity. There are few reports on such cell types. This is the case for guinea-pig megakaryocytes (Schick et al., 1984), the adipogenic cell line TA1-R6 (Reid et al., 1991) and mouse LM fibroblasts (Lambremont et al., 1978). In these previous works, the activity of Δ^5-desaturase was assayed only on $C_{20:3,n-6}$ and not on $C_{20:2,n-6}$ or $C_{20:3,n-3}$. Nevertheless, Maeda et al. (1978) found that LM cells converted $C_{20:2(11,14)}$ into $C_{20:3(5,11,14)}$, and this was also observed in CHO cells and Chang liver cells, which do not possess a Δ^6-desaturase activity. To a lesser extent, this conversion occurred also in FM3A mouse mammary cells, L5178Y mouse lymphoma and WI-38 human embryonic fibroblasts. The last three lines also exhibited a significant Δ^6-desaturase activity and a high rate of retroconversion (Maeda et al., 1978), factors that favour the normal pathway. In spite of this, the existence of the conversion of $C_{20:2(11,14)}$ into $C_{20:3(5,11,14)}$ has also been demonstrated in rat liver (Ullman and Sprecher, 1971). The significance *in vivo* of this new pathway remains at present speculative, but, as indicated by others (Cook et al., 1991), could be of importance in states of essential fatty acid deficiency. We are at present studying the effect of alternative fatty acids on the growth and differentiation of K562 cells.

In conclusion, K562 human leukaemia cells synthesize PUFA solely by an alternative pathway involving Δ^5-desaturase. These cells are a useful model to study this new pathway, since (i) they do not possess a Δ^6-desaturase activity, and (ii) the retroconversion of the main substrates of Δ^5-desaturase in the alternative pathway ($C_{20:2(11,14)}$ and $C_{20:3(11,14,17)}$) is very low. The possible physiological significance of this new route deserves further and more detailed studies.

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